SESSIONE: APPROCCI TRASLAZIONALI ALLA BIOLOGIA SPERIMENTALE

MORPHO-FUNCTIONAL MODIFICATIONS OF GUT BARRIER PROPERTIES INDUCED BY SURFACE LAYER PROTEINS (S-LAYER) FROM *LACTOBACILLUS HELVETICUS* ATCC® 15009™ IN A CO-CULTURE OF CACO2/HT-29 CELLS AS A MODEL OF HUMAN INTESTINAL EPITHELIUM.

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Healthy gut barrier (GB) can be improved by specific probiotic strains, even if their reliability and safety concerns in clinical practice are largely discussed. Paraprobiotics and postbiotics can represent a valid alternative due to their intrinsic higher stability and preservation in food matrices. Among probiotic species, Lactobacilli constitute an important group of microorganisms able to stimulate host's immune system and act as an effective therapeutic alternative for the treatment of gut inflammation, obesity, and chronic degenerative diseases.
The present study investigated the effects of surface-layer proteins (S-L) of the dairy strain *Lactobacillus helveticus* ATCC® 15009™ (*Lh* ATCC® 15009™) on the morpho-functional modulation of GB in comparison to live or heat killed *Lh* ATCC® 15009™ in a Caco-2/HT-29 70/30 co-culture cells. Live or heat-killed *Lh* ATCC® 15009™ (100 CFU/cell and 1000 CFU/cell) negatively affect transepithelial electrical resistance (TEER) and paracellular permeability, resulting in an altered distribution of tight junction (TJ) and protein Claudin-1, stained by immunofluorescence (IF). Conversely, the addition of S-L, in amounts present into the doses of *Lh* ATCC® 15009™ administered to cells, improves TEER, and decreases permeability in physiological conditions only when basal TEER registered in co-cultures established by Caco-2 and HT-29 parental cell lines with at least 40 and 21 sub cultivation passages respectively is minor than 50 ohm*cm^2. This experimental condition may suggest the presence of a physiologically leaky gut as it occurs in old people. Transmission electron microscopy (TEM) and IF analyses suggest that S-L induces a structural TJ rearrangement and desmosomes' formation and stability. S-L is also able to restore TEER and permeability of GB in the presence of lipopolysaccharide (LPS), but not of pro-inflammatory cytokines (TNF-α plus IFN-γ). IF analysis shows an increase in Claudin-1 staining when LPS and S-L were co - administered, suggesting that the downstream Toll-Like Receptor- mediated signaling (TLR4 for LPS, TLR2 for S-L) may result in junctional apparatus remodeling, such as increased desmosomes' protein complexes transcription or TJ protein phosphorylation and redistribution. In addition, S-L can counteract the reduction of alkaline phosphatase detoxification activity and the enhancement of pro-inflammatory interleukin-8 (IL-8) release both induced by LPS. Altogether, these data obtained in a model of injured intestinal epithelial barrier, either physiological or induced, corroborate the supposed paraprobiotic role of S-L from *Lh* ATCC® 15009™ as a possible candidate for therapeutic and prophylactic uses in alternative to viable microbiota, in conditions related to gastrointestinal health and extra-intestinal disorders, some of which are correlated with gut dysbiosis.

Ferraretto A. et al." Morphofunctional properties of a differentiated Caco2/HT-29 co-culture as an in vitro model of human intestinal epithelium". 2018, DOI: 10.1042/BSR20171497.
Siciliano, R. A. et al. "Paraprobiotics: A new perspective for functional foods and nutraceuticals". 2021, DOI.org/10.3390/nu13041225.